IPS Interdisciplinary Journal of Biological Sciences
IPS Interdiscip J Bio Sci, 3(1): 45-50 (2024)
DOI: https://doi.org/10.54117/iijbs.v3i1.16

Evaluating the Phytochemical, Proximate Compositions and Free Radical Scavenging Effects of *Gossypium hirsutum* Leaf

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Abstract

The therapeutic and economic value of most indigenous plants are increasingly exploited in treatment and basically, a means of lively-hood for most rural dwellers. This study evaluates the phytochemical, proximate compositions and scavenging effects of *Gossypium hirsutum* leaf extract. Phytochemical screening was carried out by quantitative phytochemical techniques, following a proximate parameters determination by quantitative estimation. The scavenging effect of the *G. hirsutum* leaf was assessed on *2,2-diphenyl-1-picrylhydrazyl* (DPPH) radical co-incubated model. Quantitative phytochemical screening revealed alkaloids (12.20 ± 0.28 mg RE/100 g), cardiac glycosides (0.99 ± 0.65 mg RE/100 g), tannins (2.73 ± 0.01 mg TAE/100 g), total phenol (50.82±0.02 mg GAE/100 g), saponins (2.63 ± 0.04 mg RE/100 g), and flavonoids (11.90 ± 0.4 mg RE/100 g). The proximate presents percentage moisture contents (8.74 ± 0.04), protein (17.58 ± 0.03), crude fat (7.10 ± 0.01), ash contents (7.22 ± 0.07), crude fibre (11.40 ± 0.01) and carbohydrate (48.04 ± 0.08), while mineral elemental analysis revealed potassium (740.28 ± 0.02 ppm), sodium (510.50±0.12 ppm), magnesium (610.82 ± 0.04 ppm), calcium (640.20 ± 0.06 ppm), iron (39.02 ± 0.06 ppm) and phosphorus (326.9 ± 0.32 ppm), respectively. The carbohydrates, proteins and fat present insinuate *G. hirsutum* leaf serves as a good source of basic daily nutritional requirements and energy. A comparative DPPH radical scavenging effect displayed by the *G. hirsutum* leaf extract (IC_{50} = 5.26.; ascorbic acid, IC_{50} = 7.36), suggests its antioxidant ability partly owing to the phytochemicals inbuilt observed under the condition of this investigation. The biological composition of *G. hirsutum* leaf may contribute to the scavenging effect, finding adequate credence in the complementary and as alternative medicine.

Keywords: *Phytochemical, Gossypium hirsutum, proximate analysis, antioxidant activity, Mineral analysis*

Article History
Received: 09 Mar 2024
Accepted: 21 Mar 2024
Published: 15 Apr 2024

Scan QR code to view•

How to cite this paper: Suleiman, M. S., Omale, J. A., Ayeni, G., Ali, B. A. ., Ojogbane, E., Sule, F. A., Jegede, R. E., & Mohammed, L. S. Phytochemical, Proximate, Mineral Analysis and Antioxidant Activity of the leaves of Gossypium hirsutum. *IPS Interdisciplinary Journal of Biological Sciences*, *3*(1), 45–50. https://doi.org/10.54117/iijbs.v3i1.16

1. Introduction

Plants formed the foundation of traditional medical systems in the biosphere. They are the primary source of novel therapeutic compounds for the creation of innovative pharmaceutics (Aziz *et al.*, 2018). The use of plants as medication, dietary supplements and foods are of increase due to the shortage of modern drugs and a non-proactive measures in the agricultural practice and approach in most developing

nations across the globe (Ahad *et al*., 2021; Ayeni *et al*., 2015; Rout *et al*., 2009).The phytochemical components such as tannins, flavonoids, alkaloids, and saponins, cardiac glycosides and others known as secondary metabolites have specific physiological effects on humans (Aborisade *et al*., 2017; Akinmoladun *et al*., 2007; Koche *et al*., 2016). Aside harboring the phytochemicals, plants are known to contain important biomolecules (carbohydrates, proteins, fat), minerals and water. These components are vital to plants' survival in terms of energy needs and metabolism. The mineral composites, like calcium, magnesium, phosphorus, iron, and potassium being highly soluble minerals with ease of bioavailability, assimilation and modulating effects, occupy a central place in human health (Soetan *et al*., 2010).They are components of the animal bones, tissues, blood, muscle, and aids cell to cell communication. Research report a synergistic nature of chemicals in these plants species unique to the central role of countering oxidative stress in order to shield the macromolecules from the free radical attacks in animals including human *(*Ponnampalam *et al*., 2022). This preventive coordination effects is probably owned to the phytochemical alignment and diversity in most indigenous medicinal plants.

Gossypium hirsutum or upland cotton, is a member of the *Malvaceae* family of plants. It is a perennial shrub which can grow to a height of 1.5 to 2 meters, with ability to bear fruits and produce flowers at any time in the year (Farooq, 2019). Investigation into the pharmacological effects of the fruits showed a wide range of activities against various diseases that include cancer, heart disease, and digestive disorders (Oni *et al*., 2022). Previous findings showed that gossypol derivative of the seeds displayed reversible infertility effects in male (Al-Snafi, 2018). *G. hirsutum* leaf extract using aqueous extraction process demonstrated moderate *in vitro* antiviral activity. Till-date, assessment regarding the chemical, minerals, proximates constituents and radical scavenging effects are not exhaustively conducted owing to its medicinal and economic value to the various area of geographical distribution. To this end, the design of this study aimed to evaluate the phytochemical, proximate compositions and radical scavenging effects of *G. hirsutum* leaf extract.

2. Materials and Methods

Chemicals and Reagents

Chemicals and reagents used for this work were of analytical grades, products of the manufacturing company BDH in England.

Plant Collection and Authentication.

Fresh leaves of *G. hirsutum* were collected at the Anyigba, Dekina Local Government Area Kogi State University. The identification and authentication of the plant was done by a plant Taxonomist at herbarium, Department of Plant Science and Biotechnology, Kogi State University, Anyigba. A voucher specimen was deposited leading to the assignment of reference number, 156. The leaves were washed and air dried for 14 days. The dried samples were stored for extraction procedure.

Preparation of the Extract of *G. hirsutum* Leaf

The dried plant parts (20 g) were pulverized to a reasonable size using modern laboratory grinding pistle. Extraction with methanol (1:5 w/v) were performed for 48 hrs. The extract mixture was separated using Whatman No. 1 filter paper. The organic solvent was recovered at 40 ºC ± 2 ºC, with the aid of a Heidolph rotary evaporatory machine. Filtrate was concentrated to extract and dried under the fume hood overnight.

2.2.1 Total Yields Estimation of the Extra

This was estimated from the ratio of weight of extract obtained to the initial sample multiply by a hundred.

$$\% \text{ Yield} = \frac{\text{Weight of extract}}{\text{Weight of pulverized leaf}} \times 100$$

Quantitative Phytochemical Screening of *G. hirsutum* Leaf Extract

Determination of Tannins

Tannins were determined by Folin-Ciocalteu method (Arabshahi-Delouee & Urooj, 2007). Approximately, 0.1 mL of the sample extract of *G. hirsutum* was added to different volumetric flask each containing 7.5mL of distilled water, 0.5 mL of Folin-Ciocalteu phenol reagent and 1 mL of 35% sodium trioxocarbonate (IV) solution and diluted to 10 mL with distilled water. Each mixture was shaken and kept at room temperature for 30 mins and then the absorbance was taken using a spectrophotometer at wavelength of 725 nm.

A blank solution to zero the spectrophotometer was prepared by the same procedure described above without using the sample extract. Tannin concentration was calculated using a reference curve of pure quercetin.

Determination of Saponins

Total saponins content was determined by method described by (Makkar *et al*., 2007). In this case, 50 µL of the sample extract of *G. hirsutum* was added to 250 µL of distilled water in different volumetric flask. To each volumetric flask 250 µL of vanillin reagent was added then 2.5 mL of 72% sulphuric acid was added and shaken well. Each resulting solution was kept in water bath at 60 ºC for 10 mins. After 10 mins, each volumetric flask was cooled in an ice water and the absorbance was taken at 544 nm.

Vanillin was prepared by adding 800 mg of vanillin in 10 mL of 99.5% ethanol solution.

A blank solution to zero the spectrophotometer was prepared by the same procedure described above without using the sample extract. Saponin concentration was calculated using a reference curve of pure quercetin.

Determination of Total Phenol

Total phenol content was determined by Folin-Ciocalteu method (Arabshahi-Delouee & Urooj, 2007). A 20µl of sample extract of *G. hirsutum* was added to 1.16 mL of distilled water and 100 µL of Folin-Ciocalteu reagent followed by addition of 300 µL of 20% Sodium trioxocarbonate (IV) solution in different volumetric flask. Each mixture was incubated in shaking incubator at 40 ºC for 30 mins and the absorbance was taken at 760 nm.

A blank solution to zero the spectrophotometer was prepared by the same procedure described above without using the sample extract. Total phenol concentration was calculated using a reference curve of pure quercetin.

Determination of Flavonoids

Total flavonoid content was determined by method described by (Arabshahi-Delouee & Urooj, 2007). It was measured by the aluminium chloride colorimetric assay. A 1 mL of sample extract was added to 4 mL of distilled water in different volumetric flask. To each volumetric flask, 0.3 mL of 5% Sodium Nitrite was added and then after 5 mins, 0.3 mL of 10% aluminium chloride was added and left for 5minutes after which 2 mL of 1M sodium hydroxide was added. Each volumetric flask was then diluted to 10 mL with distilled water and the absorbance taken at 510 nm.

A blank solution to zero the spectrophotometer was prepared by the same procedure described above without using the sample extract. Total flavonoid concentration was calculated using a reference curve of pure quercetin.

Determination of Cardiac Glycoside

Cardiac Glycoside content was determined according to (Arabshahi-Delouee & Urooj, 2007), where 10% extract of sample of *G. hirsutum* was mixed with 10 mL of Baljet reagent in different volumetric flask. After an hour, each mixture was diluted with 20 mL of distilled water and the absorbance was taken at 495 nm.

A blank solution to zero the spectrophotometer was prepared by the same procedure described above without using the sample extract. Cardiac glycoside concentration was calculated using a reference curve of pure quercetin.

Determination of Alkaloids

Alkaloids were determined by the method described by (Arabshahi-Delouee & Urooj, 2007). Fifty milliliter (50 mL) of 20% acetic acid was added to 5 g of sample extract of *G. hirsutum* in different 250 mL beakers and covered to stand for four hours (Mayer's test). Each mixture containing solution was filtered and the volume was reduced to one quarter using water bath. To each sample, concentrated ammonium hydroxide was added drop wise until the precipitate was completed. Each solution was allowed to settle, and precipitate was collected by filtration and the filter paper was weighed before and after collection of precipitate. The concentration of alkaloid was calculated using the formula:

$$\% \text{ Alkaloid} = \frac{W_2 - W_1}{\text{Weight of sample}} \times 100$$

Where W_2=Weight of Paper + Alkaloid

W_1= Weight of Paper

Determination of the Proximate Composition:

Moisture Content Determination

The moisture content was determined according to (In, 1990). Briefly, 5 g of each sample was measured and mixed thoroughly and was then transferred into a pre weighed dish. Dish and sample were weighed, dried to constant weight at 95-100 ^{o}C for 5 hrs and when drying is complete. Dish was placed in a desiccator to cool, and sample was reweighed.

$$\text{Moisture content (\%)} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

Where W_2= Weight of empty dish

W_1= Weight of dish + undried sample

W_1= Weight of dish + dried sample.

Fat Content Determination

The fat content was determined according to (In, 1990). A 250 mL clean boiling flask was dried in a Gen lab oven at 105 ^{o}C for 30 mins transferred into a desiccator to cool. Then 5 g of sample was weighed into a labelled thimble, and place inside a cooled boiling flask. The boiling flask which was filled with 300 mL of petroleum ether at boiling point of 40-60 ^{o}C, the extraction thimble was lightly plugged with cotton wool. Soxhlet apparatus was assemble and allow to reflux for 6 hrs, the thimble was removed with care and petroleum ether was collected at the top container of the setup and drain into flask for re use and when flask is almost free of the solvent. It was removed and dried at 105-110 ^{o}C for 1 hr then transferred from the oven into a desiccator and allow to cool, then weighed.

$$\text{Fat content (\%)} = \frac{W_2 - W_1}{W_3} \times 100$$

Where W_1= Weight of empty flask

W_2= Weight of flask + oil extracted

W_3= Weight of sample.

Protein Content Determination

This method of protein determination following the method of (In, 1990). The underlying principle behind the method is the estimation of the total nitrogen in the food and subsequent conversion of the Percentage of the nitrogen to protein, with the assumption that all nitrogen in food is present as Protein. Then using a conversion factor the actual percentage of nitrogen in the food is determined. The conversion is done using the formula below.

% protein = % N × F = 100/%N in food protein

Where F is the conversion factor = 6.25.

Two grams (2 g) of protein was weighed into a kjeldahl flask and 5 g of sodium sulphate and 1g of copper was added to the flask, followed by 25 mL of concentrated sulphuric acid a small glass was added to prevent bumping during heating in the fume cupboard at a temperature of 420 ^{o}C for 30 mins, it was then removed, cooled and washed to remove any black with distilled water and it was re heat until the green colour disappear, cool and it was transferred to 250 volumetric flask which was later made to the mark with distilled water then distillation and titration follows.

Determination of Carbohydrate Content

This was determined using the guidelines of (In, 1990) by direct analysis of carbohydrate. That is the sum of all other component subtracted from hundred gives the carbohydrate content. Carbohydrate content is gotten by difference after adding the protein value, ash value, dietary fibre value, fat value and moisture value the total value is subtracted from 100 the remaining value is the carbohydrate value.

Carbohydrate value = (100 - sum total of other nutrient)

Crude Fiber Determination

The crude fibre content was determined according to (In, 1990). A 5 g of the sample was defatted with petroleum ether and was boil for 30 minutes under reflux with 200 mL or a solution containing 25 g of sulphuric acid per 100 mL of solution. The solution was filtered through linen, washed with boiling water until the washing are no longer acid, then the residue was transferred to a beaker and boil for 30 mins with 200 mL per 100 mL, the final was filtered through a sieve and ignited asbestos in a gooch crucible dry in a Gen lab oven set at 105 ^{o}C for 3 hrs and weighed then incinerated, cooled, and weighed. The loss is weighed after incineration x 100 is the percentage crude fiber.

$$\text{Fibre content (\%)} = \frac{W_2 - W_1}{W_3} \times 100$$

Where W_1 = Weight of sample before incineration
W_2 = Weight of sample after incineration
W_3 = Weight of original sample.

Determination of Ash Content

The ash content was determined according to (In, 1990). A 2 g of finely ground, dry sample was weighed into a crucible, and placed on a Bunsen flame inside a fume cupboard to drive off most of the smoke then sample was transferred into a muffle furnace at 550 ^{o}C then it was allowed for two hour or until a white moisten with small among of water to dissolve salts, dry in oven and was cooled inside a desiccator and reweighed.

$$\text{Ash \%} = \frac{W_2 - W_1}{\text{Weight of sample}} \times 100$$

Where W_2= Weight of sample + crucible
W_1= Weight of sample

2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Activity

The free radical scavenging activity was followed by preparing 0.002% DPPH solution in methanol was determined using the method adopted by (Adesegun *et al*., 2010). The varying concentration of the extract was prepared in methanol, 3 mL of all the concentrate, 0.03 g of DPPH in (25, 50, 100, 200, 400, µg/mL) of the extract were mixed with 1mL of methanolic solution of DPPH. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30 mins. The absorbance was read against blank at 517 nm. Inhibition of free radical by DPPH in percent was calculated as follows:

$$\text{\% Scavenging} = \frac{(\text{Absorbance of blank} - \text{Absorbance of sample}) \times 100}{\text{Absorbance of blank}}$$

The inhibitory concentration at 50 (IC_{50}) in µg/mL was calculated by plotting percentage inhibition against the log concentration, using the equation: y = mx + c when y = 50.

Determination of Mineral Composition

The major elements, comprising calcium, sodium, potassium, magnesium and trace elements (iron and zinc) were determined according to the method implored by (Adeniyi *et al*., 2012)

Statistical Analysis:

Results are presented as mean ± standard error of mean (SEM) of three replicates (n=3). Raw data were averaged and converted to SEM using GraphPad Prism version 8.0.1 for Windows, GraphPad, Software, La Jolla California. The inhibitory concentration expressed as IC_{50} values were obtained using excel by plotting percentage inhibition against the log concentration.

Results

The percentage yields of *G. hirsutum* leaf extract was estimated (25.20%). Phytochemical analysis in the current study showed alkaloids (12.20 ± 0.28), cardiac glycosides (0.986 ± 0.65), saponins (2.63 ± 0.04), flavonoids (11.90 ± 0.4), and tannins (2.73 ± 0.01 mg/100g) and total phenol (50.82 ± 0.02) and their corresponding compositions in the *G. hirsutum* leaf extract (**Table 1**). Moisture contents (8.74 ± 0.04), protein (17.58 ± 0.03), crude fat (7.10 ± 0.01), ash content (7.22 ± 0.07), crude fibre (11.40 ± 0.01) and carbohydrate (48.04 ± 0.08) were estimated as proximate components of the extract (**Table 2**). Elemental analysis revealed potassium (740.28 ± 0.01), sodium (510.50 ± 0.12), magnesium (610.82±0.04), calcium (640.20 ± 0.06), iron (39.02 ± 0.06) and phosphorus (326.9 ± 0.32) composites of the extract (**Table 3**). The biological activity of the *G. hirsutum* leaf extract revealed a comparative DPPH radical scavenging effects with IC_{50} (µg/mL) = 7.36, relative to the synthetic antioxidant compound (ascorbic acid; IC_{50} (µg/mL) = 5.26) (**Table 4**).

Table 1: Phytochemical composition of *G. hirsutum* leaf extract

Phytochemicals	Composition
Alkaloids (mg RE/100 g)	12.20 ± 0.28
Cardiac Glycosides (mg RE/100 g)	0.99 ± 0.65
Tannins (mg TAE/100 g)	2.73 ± 0.01
Total phenol (mg GAE/100 g)	50.82 ± 0.02
Saponins (mg RE/100 g)	2.63 ± 0.04
Flavonoids (mg RE/100 g)	11.90 ± 0.4

Results are expressed as mean ± SEM (*n* = 3). GAE = Gallic acid equivalent; TAE = Tannic acid equivalents; RE = Rutin equivalent.

Table 2: Proximate composition of *G. hirsutum* leaf extract

Parameters	Composition (%)
Moisture content	8.74 ± 0.04
Protein	17.58 ± 0.03
Crude fat	7.10 ± 0.01
Ash content	7.22 ± 0.07
Crude fibre	11.40 ± 0.01
Carbohydrate	48.04 ± 0.08

Results are expressed as mean ± SEM (*n* = 3).

Table 3. Mineral composition of *G. hirsutum* leaf extract

Mineral Elements	Composition (ppm)
Potassium	740.28±0.01
Sodium	510.50±0.12
Magnesium	610.82±0.04
Calcium	640.20±0.06
Iron	39.02±0.06
Phosphorus	326.9±0.32

Results are expressed as Mean ± SEM (n = 3).

Table 4: Percentage (%) DPPH inhibition of *G. hirsutum* leaf extract

Concentration (µg/mL)	(%) DPPH inhibition	
	Ascorbic acid	*G. hirsutum*
25	53.2	43.7
50	63.3	57.3
100	75.1	66.1
200	86.02	70.4
400	97.14	79.2
IC_{50}	**5.26**	**7.36**

Values are presented in IC_{50} (µg/mL), derived from the corresponding percentage inhibitions.

Discussion

Plants are by endowment a considerable reserve of therapeutic agents with increasing applications cutting across medicine, nanotechnology, industrial, agricultural and other life endeavours (Wani et al., 2022). In this study the presence of alkaloids, cardiac glycosides, saponins, flavonoids, and tannins and total phenol quantified portends the medicinal value of *G. hirsutum* leaf. Studies reveal the biological interactions of these phytochemicals with respect to their precise pharmacological actions in disease states (Msomi *et al*., 2019; Nwozo *et al*., 2023; Sanni *et al*., 2018). They are known to elicit actions on specific metabolic pathways through the modulatory actions of either the hormonal, enzymatic or the flow of metabolites with overall therapeutic effects in the biological system (Lecomte *et al*., 2017). By these phytochemical actions, biological entities are preserved, functional and maintain cellular balance. The presence of moisture contents, protein, crude fat, crude fibre, carbohydrates and ash contents confirmed the metabolic activities unique to the plant survival, amino acid precursor for synthesis and turnover rate in plant, energy production and consumption and mineral elements (Millikan, 2012). Estimation of potassium, sodium, magnesium, calcium, iron and phosphorus in this plant suggests it a source of intra and intercellular mediator of ionic transport, cofactor, haeme production and healthy tissue (Fiorentini *et al*., 2021). A high amount of calcium relative to other elements in part may be due to the contractile effect exerted by the plant in mitigating effects of drought and mechanical damage (Reddy & Reddy, 2001). This could translate by implication that consumption of *G. hirsutum* leaf might in a way boost the plasma calcium contents. The high radical (DPPH) scavenging effect of *G. hirsutum* leaf demonstrates its antioxidant status in the incubation system. DPPH has been shown to be a stable free radical. The sensitivity of this radical to a promising antioxidant compound was reported (Parcheta *et al*., 2021; Shah *et al*., 2006). The previous report was similar to the current findings in this study.

Conclusion

This study has provides insights on the phytochemical, proximate configuration and scavenging effects of *G. hirsutum* leaf. This presumably further attests to its energy yielding ability and suggestive an enzymatic activator and a promising antioxidative agents by virtues of the preliminary assessment conducted in this study. These findings could be used as rudimentary data for a functional food formulation with adequate application in nutriceutics. Assessment of the *in vivo* antioxidative effects of *G. hirsutum* leaf and a lead compound deriving the biological action(s) is recommended for future investigation

Conclusion

The whole study revealed that the sampling sites are reservoirs of crude oil degrading bacteria. The isolates possess catabolic genes which are plasmid and chromosomally mediated. Neutral pH, 35 ℃ temperature and 3 % crude oil concentration were found to be optimum conditions. The degrading potentials of these isolates could be exploited in crude oil and hydrocarbons biodegradations.

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